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TISSUE RESPIRATION IN THE LIGHT OF RECENT RESEARCH

BY DAVID FRASER HARRIS, M.D., D.Sc., F.R.S.E.

Professor of Physiology and Histology in Dalhousie University, Halifax, N.S.

IT has been known for a long time that living tissues possess both reducing and oxidising powers. In 1883 Hoppe-Seyler drew attention to the strong reducing processes in living tissues; and Professor Theobald Smith has recently used liver-juice as the agent with which to close the open end of a tube where bacteria could best grow under anærobic conditions. Ever since the time of Lavoisier it has been certainly known that the carbon dioxide and water eliminated from the animal body have been produced by the oxidation of carbon and hydrogen within it. It was originally held that this "carbonaceous" oxidation took place in the blood itself, but the undoubted production of carbon dioxide by a frog whose blood had been replaced with salt solution showed that at least that gas must have originated in the tissues and not in the blood. Oxidation and reduction evidently go on side by side within the living tissues; oxygen they must have, and they soon die if it is withheld. The source of this oxygen is of course the respiratory pigment oxy-hæmoglobin, whose loosely held oxygen is removed by the tissues which are, therefore, said to have oxygen-avidity (*Sauerstoff-Bedürfniss*) or reducing power. This continual oxidation of materials within the tissues and the reduction of the oxyhæmoglobin in the circumambient blood is conveniently called tissue-respiration. Within the last few years, attempts have been made to gain a clearer insight into both the processes of tissue oxidation and tissue reduction, with the result that both are now thought of as carried out by intracellular ferments. Many workers on the continent of Europe and in England have studied the action of what have been called oxidases, ferments believed to be concerned in effecting oxidations of a large number of substances inside the living cells. These workers have studied the expiratory phase of tissue-res-

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piration, the exact nature of the oxidative process leading to the final formation of carbon dioxide and water with the production of correlated intermediate substances.

Those who work on oxidases are continually meeting with evidences of substances in the living tissues which appear to be working in the direction opposite to that of oxidation. One particular oxidase has the power of oxidising indo-phenol-white to indophenol-blue, and has been studied by Vernon who calls it "indophenol-oxidase." Studying the quantitative estimation of this oxidase, Vernon encountered "the unavoidable presence of reducing substances some of which are possibly enzymes or reductases which act in direct antagonism to the oxidases, and under certain conditions entirely overpower them. Hence the absence of an oxidising action cannot be held to indicate the absence of oxidase unless the conditions are so chosen to give the oxidase the best possible chance of exerting its activity." Now it is just these reducing agents which, on the other hand, I have been studying for some years past. In 1885 Paul Ehrlich published an elaborate research into the reducing power of living organs whereby they were able to reduce indo-phenol-blue to the leuco-compound and alizarine blue to alizarine white. The pigments were injected subcutaneously into living animals. Ehrlich found that almost all organs examined reduced one or other of these pigments, some organs with great energy, such as liver, fat and the gastric mucous membrane. He recognized that, even when he could not detect reduced pigment, it did not prove that there had been no reduction, but only that oxidation had been quantitatively greater. The title of Ehrlich's paper was "The oxygen-avidity of the organism," for he recognised that it was in virtue of the avidity for oxygen on the part of the tissues that they were also able to reduce certain pigments to the colourless or chromogenic condition. In other words, the oxygen avidity is one expression of reducing power. Ehrlich made no suggestion that this power was due to a ferment.

In 1896 I noticed that when an animal, still alive though chloroformed, had been injected with the mixture of gelatine and soluble Prussian blue so much used by histologists for demonstrating microscopic blood-vessels, and had been cut up immediately, that such an organ as the liver, instead of being blue, was colourless. On cutting up the liver and exposing the portions to the air, the blue colour was observed to be restored until one could see minute vessels which a moment before were quite invisible. The restoration of blue colour was very rapidly brought about by pouring hydrogen peroxide

over the colourless surfaces. The bleaching of the soluble Prussian blue in the gelatine injection mass was attributed to *reduction* of the blue potassium ferri-ferrocyanide to the colourless di-potassium ferro-ferrocyanide; in terms of physical chemistry to the removal of a positive ionic charge of electricity from the tri-valent ferric-ion. Similar results were obtained on injecting the vascular system of the surviving kidney. It then occurred to me that if the kidney could reduce a pigment which was still in its vessels, the organ, if injected under sufficient pressure, might be constrained to excrete an artificial urine through the normal channel of the ureter. I found it possible to effect this. On injecting into the artery of a sheep's kidney the warm soluble Prussian blue and gelatine mixture, I obtained from the cannulated ureter a few drops of an absolutely colourless substance—an artificial urine—which on being treated with hydrogen peroxide at once became blue. The kidney had, then, excreted some gelatine and reduced soluble Prussian blue, proving that these substances had travelled from the blood capillaries to the ureter and in the passage had been reduced by the still living renal epithelium. But after a time it was noticed that the outflow from the ureter had become blue, the kidney cells had become poisoned and so no longer able to carry on their vital reduction. It is not to be supposed that the living tissues can withstand for more than a certain time treatment with substances which cannot be other than ultimately toxic for them. In later experiments ferric chloride was used with both the liver and kidney in order to determine whether a substance devoid of oxygen could be reduced to the lower form—ferrous chloride—on being perfused through surviving organs. From the kidney was obtained an artificial urine which contained ferrous chloride, and some ferrous chloride was present in the liquid which emerged from the renal vein. Similar results were got with the liver; from its bile duct ferrous chloride was drawn off (artificial bile) and in the fluid from the hepatic vein some ferrous chloride was present.

The activities of tissues may be studied in other ways than by injecting chemical substances into their vessels; for instance the organs may be crushed in a juice-press until thoroughly disintegrated, and the resulting juice mixed with some pigment or other substance the reduction of which is expected. By this means the active reducing material is brought into a contact with the reducible material which is very much more intimate than when, for instance, masses of the organ are merely immersed in the reducible solutions. By this technique, press-juice of liver and kidney of cat, sheep,

rabbit, horse and frog was able to reduce methylene blue to methylene white, sodium indigo-disulphonate to the colourless chromogen and sodium nitrate to sodium nitrite. It was found also that e.g., liver juice could reduce the pigment methæmoglobin first to the stage of oxyhæmoglobin and later to that of fully reduced hæmoglobin. A boiled control of these juices had no reducing power whatever.

Two French workers, Abelous and Gerard, as long ago as 1899 had suggested that these reducing powers of tissues might be due to the presence of a ferment to which they gave the rather barbarous name "reductase." Later reasons will be given for suggesting a more specific term for this tissue-ferment.

My work carried out in 1909-10 was undertaken with a view to determine what was the evidence for the existence of a reducing enzyme in tissue press-juice. The results then obtained, taken in conjunction with others arrived at more recently, have gone far to convince me that there is a tissue-ferment with reducing powers. We have no evidence that this ferment differs qualitatively whether it is derived from liver, kidney or other tissue.

Some of the evidence for this conclusion may be summarized as follows:

In the first place in a control experiment where the juice is boiled, none of the reducible substances mentioned above is reduced thereby. The temperature of boiling water, as is well known, destroys the activity of all enzymes.

In the next place, the general behaviour of the juice according as the temperature is raised or lowered is in agreement with the behaviour of acknowledged enzymes. Thus at minus 10°C., there is no reduction of soluble Prussian blue by fresh liver juice, and it is extremely slow at zero; inhibition may be induced indefinitely by keeping the mixture of pigment and juice surrounded by a freezing mixture; on the mixture being removed to air temperature, reduction goes on as rapidly as is usual for that temperature; the ferment, therefore, has been inhibited but not destroyed. As the temperature rises, the velocity of reduction increases correspondingly; the optimum temperature is somewhere between 40 and 45°C. Like recognized enzymes, reductase has a destruction-temperature which is in the neighbourhood of 70°C.

While fresh juice reduces soluble Prussian blue within a minute or so at room temperature (17°C.), its activity rapidly falls off, so that after twenty-four hours it takes some minutes longer to bleach the pigment; yet juice which takes some minutes at room tempera-

ture has its time distinctly shortened at 40°C., the blood heat. There is a decay in the activity of tissue reductase the longer the juice is kept even when it has been covered with a layer of toluene to prevent putrefaction. In a particular series of recent observations extending over a week, the following fall off in activity of reduction of liver juice was estimated. At the end of twenty-four hours the activity had fallen to 80 per cent. of its original value, at the end of the second day to 66 per cent., at the end of the fourth day to 30 per cent., and at the end of the eighth day to about 5 per cent. The survival of hepatic reductase to the eighth day is evidently not an isolated phenomenon, for quite recently it has been found that both hepatic xanthinase and uricase are active in liver juice as late as the fifth day. We shall later see that this decrease in activity is amenable to mathematical treatment.

Since several substances are known to be able to bleach soluble Prussian blue or cause it to fade, one had to eliminate the action of such as could possibly vitiate the results. Alkalies had first to be disposed of. It is of course true that alkalies can cause rapid fading of soluble Prussian blue and certain other pigments, but none of these is present in the living tissues. When all the various inorganic salts present in the blood or lymph had been examined, it was found that none of them caused any fading of the blue beyond what a similar dilution with water would have done. No more effective were mixtures of the salts; and Ringer's solution itself produced no fading. Reductase certainly acts like a reducer in an alkaline medium. Acid, therefore, added to the soluble Prussian blue and gelatine mixture prevents that complete reduction in the capillaries of an injected organ which occurs in its absence. Histologists recommend acetic acid being added to this particular injection mixture in order to prevent "fading by the alkaline tissues." That the inorganic salts of the blood do not reduce soluble Prussian blue is shown by the fact that when the blue and the red (of the blood) meet in the large vessels they form purple in those cases where the blood is not washed out previous to injection; but if the blue were reduced to the colourless state, the blood would be red in the large vessels, whereas it is always purple when the one pigment does not predominate over the other. It is hardly necessary to say that reduction was not due to products of putrefaction, for not only were the juices kept under toluene, but the reducing power falls off with age while the products of putrefaction must necessarily accumulate as time goes on. The next factor which had to be eliminated was the supposed reducing power of

proteins (colloids): this was taken up by a co-worker of mine, Dr. H. J. M. Creighton.* Briefly stated his conclusions were: colloids such as gelatine and egg-white reduce soluble Prussian blue with great rapidity at 100°C, in about half an hour at 60°C, while the reduction is barely perceptible at room temperature at the end of many hours. It was shown that the protein formed a colourless compound with the pigment, and that reduction was due to the removal of a positive ionic charge. Seeing, then, that fresh liver or kidney juice at room temperature can reduce soluble Prussian blue to the leuco condition within sixty seconds, the agent operative in the case of colloidal reduction is not that which we have been investigating in tissue-juices.

We think it is possible that these colloidal phenomena worked out by Creighton are the reductions which Heffter has studied. Heffter holds that the so-called reductase reductions are not vital (enzymic) but are all due to the interaction of colloids and pigments. He says that crystallised egg-albumen can effect reduction. The blood-proteins certainly cannot do so either at room temperature or at 40°C.

Now, however interesting and important the study of the action of reductase on various pigments and other salts capable of reduction may be, we have to remember that none of them is even approximately the natural medium of the tissues and most of them are distinctly toxic for the living substance. Nothing other than oxyhæmoglobin is the natural substance yielding the oxygen dealt with by tissue reductase. Dr. Creighton and I have recently, therefore, made a systematic investigation into the relationships of reductase and oxyhæmoglobin in solution, a research which has brought to light many fresh data. The method used was spectroscopic: that is, the change from the two-banded spectrum of oxyhæmoglobin to the one-banded spectrum of fully reduced hæmoglobin was followed with a direct-vision spectroscope. There are two advantages in this method; the first that the reductase was acting, as it were, in its own proper substrate, the respiratory pigment oxyhæmoglobin, the second that the end-point was as accurate as could be obtained in a spectroscopic method. The personal factor was practically eliminated. As the mixture of juice and diluted blood was examined from moment to moment, the two bands were seen gradually to fade away and be replaced by the single fainter band of the reduced pigment. The change of colour

* Creighton, H. J. M., Nova Scotian Inst. Sci., 13, (2), p. 61, (1911-12).

seen by the naked eye was observed to correspond very closely with the spectroscopic appearances. The fresh mixture of tissue-juice and oxyhæmoglobin was of course pink, but as reduction proceeded it became of a duller red, until finally when fully reduced it was of the purple or livid colour so characteristic of venous blood. Towards the end of our work we were able to say, even by inspection, when a specimen was fully reduced: the spectroscope almost always corroborated us. We found that the fresh liver juice (cat) in presence of an aqueous solution of oxyhæmoglobin (cat's blood diluted one in twenty-five) would completely reduce the pigment at 40°C. within five to six minutes. At room temperature (17-20°C.) the time was approximately four times as long. The acceleration in the rate of the reduction of blood pigment with rise of temperature was particularly instructive with fresh juice; the times of reduction were 36 minutes at 10°, 22 minutes at 20°, 10 minutes at 30°, 5 minutes at 40°, 2·5 minutes at 50°, and 1·75 minutes at 55°C.

Since reductase thus can work through a large range of temperatures, we might expect that it would be found both in cold-blooded and in warm-blooded animals. This was discovered to be the case, for we had unmistakable evidence of its activity in tissue-juices both from the frog and from the fish. At room temperature, a specimen of liver juice from the fish which reduced oxyhæmoglobin in 7 minutes, reduced it at 40°C. in 2 minutes. We found reductase in fact in four out of the five great groups of the Vertebrata: mammals, birds, amphibia and fishes. The reductase from fish's liver was amongst the most energetic of any we encountered: the reason seems clear; the fish has access to so little oxygen that its organs must be able to extract it very thoroughly.

The marked acceleration of reduction at temperatures above 40°C. is in accordance with what we know as to the intensification of respiratory tissue changes in fever. Herter has told us that in hog cholera the reduction processes are exaggerated.

We had no evidence that reductase was qualitatively different in the various organs of the same animal nor in the different kinds of animals examined. There is no specificity of reductase from any one source in reference to hæmoglobin from any other. Thus the cat's liver juice can reduce the blood of any other mammal or of a bird, a frog, or a fish. The reductase of a bird can reduce the blood of a mammal, a frog, or a fish and so on We have called these "crossed reductions;" they prove there is no mutual specificity of relationship between the enzyme and the pigment. Incidentally they corroborate the belief that the resemblances between the

hæmoglobins from various animals are more numerous than the differences. The physiological significance of the absence of any specificity between reductase and hæmoglobin is that foreign blood introduced into any animal can still be reduced by that animal so that, for instance, human tissues can obtain oxygen from the hæmoglobin of any lower animal. The danger to the human being as regards blood-transfusion is not that the foreign blood will not be reduced, but that it may act hæmolytically toward the red corpuscles of the receiver.

The object of the tissue reduction of oxyhæmoglobin is virtually to cause the oxygen to dissociate from the pigment; all work therefore on the subject of the dissociation of oxyhæmoglobin has a bearing on the present problem. Some workers have laid stress on rise of temperature as a factor in this dissociation. Where this factor is operative, it is a vastly slower action than that of reductase. After many hours a tube of oxyhæmoglobin kept at 40°C. is still unreduced, whereas, as we have seen, certain juices at room temperature will reduce twice or more of their volume of diluted blood in two to three minutes. Temperatures above body-temperature (40°C.) do not enter into the problem in healthy animals. Since oxyhæmoglobin can be slowly reduced at 10°C. and even at 0°C., we hold that reductase is the factor operative at low temperatures in the cold-blooded animals. The almost complete cessation of reduction at 0°C. and below is an interesting demonstration in vitro of the artificial counterpart of that cessation of tissue respiration which constitutes the condition known as "latent life."

In the next place the presence of carbon dioxide in the blood has been proved by Barcroft to be a factor in facilitating the dissociation of oxyhæmoglobin in vitro. This is regarded as a most important factor in the case of cold-blooded animals. Important as this has been shown to be in laboratory experiments, we are fully convinced that it is not the chief factor in the reduction of oxyhæmoglobin even in the poikilothermic animals. The factor responsible for the reduction of oxyhæmoglobin is highly insoluble; but carbon dioxide is very soluble. Similarly, traces of acid have in laboratory experiments been demonstrated to facilitate the separation of oxygen from oxyhæmoglobin. We do not think that this either is a factor of high importance in tissue reduction. Since it is true that juice kept aseptic developes acidity in autolysis, the older the juice the more vigorously it ought to reduce if acid were an important factor, but we have shown that the exact opposite is the case. Traces of acid tend to form methæmoglobin, a pigment

we have never noticed in any mixture of active tissue-juice and oxyhæmoglobin. Again the acids in question—for instance lactic—are soluble, the reducing agent in press-juice is comparatively insoluble. It might be noted that in dealing with liver juice and oxyhæmoglobin, we have eliminated both bile and dextrose as factors in the reduction of the pigment. It may be remarked that the so-called reducing power of colloids is exerted only against certain pigmentary substances and not at all against oxyhæmoglobin. In other words, the "Creighton effects" have no analogies in connexion with the reduction of oxyhæmoglobin; for one thing, it is impossible to heat blood to 100°C. without its being decomposed.

If the substance responsible for reduction in tissue-juices is an enzyme, it ought to be injuriously affected by contact with poisons, substances known to destroy or retard the action of catalysts in general. A considerable number of such substances were examined by allowing fresh liver juice (cat) to remain in contact with solutions of the poison for ten minutes, and then comparing the time required by the poisoned juice to reduce oxyhæmoglobin with that required by the same quantity of unpoisoned juice. Two strengths of poison were employed; 0·1 molar and 0·01 molar, a strong and a weak respectively. All the following were investigated: formaldehyde, mercuric chloride, potassium cyanide, gold chloride, osmic acid, manganous chloride, ammonium bromide, arsenious acid, ammonium chloride and sodium arsenite. Unfortunately, certain toxic substances could not be used at all on account of the way in which they caused the blood solution to fade when added to it; among such were acids, copper sulphate, etc. In a particular series of experiments, ten minutes was the time found to be necessary for the complete reduction of oxyhæmoglobin by unpoisoned juice, whereas the times for poisoned juice were with the weaker solutions as follow: arsenious acid 33′, potassium cyanide 30′, mercuric chloride and sodium arsenite 17′, gold chloride 15′, osmic acid 13′ and formaldehyde 10′. When the stronger solutions were employed, the times were lengthened, for instance, for formaldehyde 48′, potassium cyanide 34′, manganous chloride 25′ and osmic acid 19′. Ammonium chloride alone of all the substances tried had no poisonous effect at either concentration; this is in accordance with what we know of it therapeutically.

One substance highly poisonous to animals, carbon monoxide, is of particular interest spectroscopically. It is a poison, because it unites so firmly with hæmoglobin that it prevents the formation in the lungs of the much less firm combination, oxygen and hæmo-

globin. The pigment therefore carries carbon monoxide instead of oxygen to the tissues which are, in consequence, starved of oxygen or asphyxiated. The affinity of carbon monoxide for hæmoglobin is stronger than that of carbon monoxide for the tissues. Translated into terms of our conception, the tissues cannot split off the carbon monoxide from the hæmoglobin because reductase being an oxygen carrier and oxygen activator has no affinity for carbon monoxide. It was, therefore, very interesting to determine whether the carbon-monoxide-hæmoglobin, a pigment with a well-known spectrum, would remain unaltered in the presence of reductase or whether it would be in any way changed. It remained unaltered for many hours at 40°C., showing that reductase in its state of comparative freedom in tissue-juice was as powerless to break up the carbon-monoxide-hæmoglobin union as it is in intact cells.

Recently we have studied the enzymic nature of the active agent of tissue-juice from the kinetic standpoint. Measurements were made to determine the value of the temperature coefficient of the activity of reductase, and also to determine the nature of the law governing the decay in the activity of the enzyme. As regards the former, we obtained the necessary data from experiments on the reduction of oxyhæmoglobin by cat's liver juice at different temperatures. To determine the temperature coefficient, the time required to reduce oxyhæmoglobin at any one temperature was divided by the time required to reduce it at a temperature 10° higher. Between 10° and 40°C. the velocity of reduction is approximately doubled for each 10° rise in temperature, so that the temperature coefficient is about two. This discovery as to the behaviour of tissue-juice with rise of temperature, confirms our general contention that we are dealing with an enzyme. Above 40° it has been found that the increase in the velocity of reduction with rise in temperature rapidly falls off. Between 50° and 60°C., the temperature coefficient has been found to be 1·43. Although usually the temperature coefficients of reactions decrease slightly with increase of temperature, the decrease in the values obtained for the reduction of oxyhæmoglobin by reductase at temperatures above 40°C. is much greater than would be the case in ordinary chemical reactions. Since it is exceedingly probable that the optimum temperature of reductase lies between 40° and 46°C., the acceleration of the velocity of reduction due to increase in temperature is evidently to a certain extent counteracted by a partial inhibition or destruction of the enzyme, the result being a decrease in the value of the temperature coefficient.

Mathematical treatment of our data shows that if the decrease in activity of the enzyme be regarded as proportional to the decrease in its reducing power, then the expression

$$\frac{1}{0.4343t}.\log\left(\frac{a}{a-x}\right)=k$$

is found to hold. Considering the sources of error in our experiments, the values obtained for k are sufficiently constant to warrant our assuming that the decay in the activity of reductase follows the monomolecular or logarithmic law. In the foregoing equation a represents the initial activity (100 per cent.) of the enzyme and x the percentage decrease in activity at the end of time t. Determinations of the reducing power of cat's liver juice of different ages, carried out at 55°, 50°, and 40°C., gave the following respective mean values for k, 0·0132, 0·0134 and 0·0121.

We are now perhaps in a position to summarize the evidence which has been accumulating to indicate that in living tissues there is a ferment for internal respiration capable of effecting chemical reduction.

1. The criterion of solubility naturally occurs to one first of all. Reductase is certainly not soluble if by "soluble" we mean capable of entering into pure water. We have, however, found that with difficulty some of it can pass from liver juice into 0·75 per cent. NaCl and from disintegrated muscle into glycerine and saline solution, which mixture seems better than either menstruum alone. Reductase is not soluble in the sense that pepsin is soluble; it leaves its association with the cell proteins with great difficulty, nor will it dialyse away from them. The glycerine and saline extract (muscle) or "solution" did, however, reduce oxyhæmoglobin in two to three minutes, while the boiled control had no effect whatever. Neither reagent by itself has any reducing effect. This glycerine and saline muscle extract also reduced soluble Prussian blue.

All subsequent attempts to isolate the ferment have failed. Indeed, it was through finding the injurious influence of alcohol and ammonium sulphate on the ferment used with the view of precipitating it, that we were led to study the poisonous effects of other materials. The comparative insolubility of reductase is perfectly intelligible. The rôle of the ferment is to obtain activated oxygen at the boundary of the cell; it would not serve the interests of internal respiration for reductase to be able to leave the cell and circulate in the blood. It is not a secretion like the exo-

enzymes pepsin, ptyalin, etc.; it is an endo-enzyme as is glycogenase, but, unlike glycogenase, it is insoluble, and as such by no means alone in that class.

2. The next criterion that may be applied is the manner in which the activity of an enzyme varies with fluctuation of the temperature. The behaviour of reductase is wholly in accordance with that of undoubted enzymes; there is inhibition but not destruction below and at 0°C., as the temperature rises there is greater and greater velocity of action until the destructive effects of heat begin to make themselves felt. That is to say, there is an optimum and there is a destruction temperature

3. The temperature coefficient of, approximately, two between 10° and 40°C. is in line with known enzymic action.

4. This is also true of the logarithmic nature of the decay in the activity of the enzyme.

5. The deterioration with age of reductase in a moist medium also conforms to the behaviour of other enzymes. It withstands complete dessication badly.

6. The fact that poisons for catalysts similarly affect the actively reducing substance in press-juice, is in favour of that substance also being an enzyme.

7. The criterion of reversibility is one which is difficult to to apply to reductase. Stated baldly, the ferment does not induce any reversed action in the direction of oxidation. Press-juices reduce materials once for all, and no oxidation in virtue of the presence of reducing agents is possible. Oxyhæmoglobin once reduced is not re-oxidised. But since oxidases are always present acting simultaneously with reductases, the chemical complex oxidase-reductase is functionally equivalent to a reversible ferment. In this connexion, one should remember that the Canniszaro reaction—simultaneous oxidation and reduction—has actually been obtained when certain aldehydes were digested with liver tissue, thus

$$2R\cdot CHO + H_2O = R\cdot CH_2\cdot OH + R\cdot CO\cdot OH$$

where one molecule of the aldehyde is oxidised to the acid and the other reduced to the alcohol. Parnas has actually suggested the term "aldehydemutase" for the hypothetical enzyme concerned.

The object of tissue respiration as distinct from tissue nutrition may be said to be two-fold; to produce heat and to prepare katabolites for excretion. Thus carbohydrates are oxidised to yield carbon dioxide and water as end-products, although exactly how is not even yet throughly understood. As aldehydes (aldoses)

the carbohydrate chains are capable of disintegrative oxidation; and already aldehydases have been assumed.

The fatty acids are certainly oxidised in the tissues after being desaturated in the liver. The long carbon chains are broken up at the β-carbon atom on each oxidation until finally β-hydroxybutyric acid is obtained which is ultimately oxidised in the presence of sufficient carbohydrate to carbon dioxide and water. If insufficient carbohydrate be present, then the acetone group of bodies is excreted which is the chemical abnormality in diabetes.

Amino-acids are certainly oxidised after deamination which is itself regarded as enzymic. Amino-acids thus deaminated are ordinary fatty acids which can then undergo the progressive oxidative break-down to which these acids are liable. Lastly, bodies of the purin group undergo oxidations within the tissues, and specific enzymes are described which accomplish these.

These various oxidations in the tissues which are now regarded as enzymic are thermogenetic and are entitled to be considered as making up internal respiration on its dis-assimilatory side. We may in fact, speak of tissue-expiration, since the various processes have for their object the excretion of waste-products. The consensus of chemical opinion, then, is that tissue-*ex*piration is dominated by intracellular ferments, and all that we claim at present is that tissue-*in*spiration should be so regarded. We have evidence of a very powerful intracellular ferment which brings active oxygen within the sphere of the oxidases. This is the ferment already called reductase in 1899. I think that the time has come to give it its specific name of *hæmoglobinase*, for hæmoglobin is the source of the oxygen it deals with, hæmoglobin is the substance reduced by it, hæmoglobin is its natural substrate or substance on which it normally acts.

A comparatively recent observation by Dakin* is of interest in this connexion. "The oxidation of β-hydroxybutyric acid to acetoacetic acid was shown by Dakin and Wakeman to be due to an enzyme which could be roughly separated from liver-tissue. The action of the enzyme was not very vigorous, but was markedly increased by the presence of oxyhæmoglobin. Oxyhæmoglobin alone was entirely without action." It seems exceedingly probable that in separating, admittedly roughly, the oxidising enzyme, a certain quantity of reductase was present along with the oxidase and added hæmoglobin. The reductase would reduce the oxyhæmo-

* Dakin, "Oxidations and reductions in the animal body." Dakin, Longmans, 1912, p. 23.

globin activating the oxygen which under the influence of the oxidase oxidised the acid as reported. This explanation at least accounts for the phenomenon observed and especially for the fact that the hæmoglobin *alone* had no influence.

In his recent text-book of "Physiology," Professor Starling* writes: "There is no doubt that reducing substances are found under normal circumstances in the tissues . . . . and it is possible that such reducing substances may aid i t activating oxygen and in the *induction* of certain oxidative processes." The italics are mine, for this precisely expresses my belief that the chief reducing substance in fresh normal tissues is an enzyme which does originate or induce the oxidative tissue activities. This was indeed Hoppe-Seyler's original suggestion; all our work is in the direction of confirming it.

The source of active oxygen, as is well known, has been referred to intracellular peroxides of which an inorganic example is hydrogen peroxide. Now it is remarkable that most tissue-juices contain the enzyme catalase whose duty would seem to be to decompose hydrogen peroxide into water and molecular oxygen thus—

$$2H_2O_2 = 2\ (H_2O) + O_2;$$

but that catalase is not responsible for the reductions we have been studying we have detailed evidence. A peroxidase has been postulated as responsible for the decomposition of hydrogen peroxide which yields the active oxygen thus—

$$H_2O_2 = H_2O + O'.$$

Whatever, then, the peroxidase is, it is virtually a reducing agent. I am not prepared to explain the relationship of reductase to peroxidase or to the hypothetical peroxides, but I prefer to conceive of the inspiratory phase of tissue-respiration as the removal of oxygen from cell lymph and, therefore, ultimately from oxy-hæmoglobin by the activity of a reducing endo-enzyme. I find in tissues an enzymic reducer; it reduces hæmoglobin to the completely reduced condition, and since it carries oxygen from it in this way it is, in one sense, an oxygenase. It is quite possible that the organic peroxide plus ferment of certain writers is none other than our hæmoglobinase which is certainly of colloidal nature. There seems no need of retaining the term "oxygenase" when hæmoglobinase is a more specific term for a ferment which can do all that oxygenase is supposed to do and considerably more.

We have seen that reductase is very insoluble: the true meaning of this may be that what we call reductase is in ultimate cellular

* P. 1236.

analysis the totality of certain side-chains of the living protoplasmic molecule (biogen) which possess affinities for oxygen. These, in the nature of things, cannot be disrupted from the biogen without compromising its functional integrity. This sort of thing on ultimate analysis an *endo*-enzyme proves itself to be; and Vernon has indeed remarked on the insolubility of certain oxidases.

It is true that on this view the distinction between "vital protoplasmic activities" and enzymes is obliterated, but it is quite possible that that distinction has been made too absolute. When an exo-enzyme or enzymic secretion (such as ptyalin, pepsin) can perform its function equally well in the cavity of a viscus or in vitro we may be justified in maintaining the distinction between vitality and enzymic action. The secretion enzyme was, however, part of the protoplasmic molecule before its separation. It is not the act of disrupting the side-chain that constitutes an enzyme; doubtless disrupted side-chains are our separable ferments and because disrupted are more or less soluble. But non-separated side-chains can still be called ferments (endo-enzymes) which because undisrupted are "insoluble." The former—the secretion-enzymes—are destined to leave the parent protoplasm, the latter are not intended to be separated from the biogens and are, therefore, called endo-enzymes. It is not whether they act outside or inside the protoplasm that constitutes them ferments, it is their functional powers that confer the title on them. Since animal heat is genetically an intracellular affair, the ferments, therefore, which are concerned in its evolution, hæmoglobinase and the oxidases, are also intracellular. The former is non-specific, the latter highly specific: the former is for obtaining oxygen wherever available: the latter are specialized each for the oxidation of only one kind of substance.

But reductase is not merely a deoxidiser: although in Nature it is concerned only with the reduction of oxyhæmoglobin, yet it is a true reducer; it can reduce substances as stable as soluble Prussian blue; alizarine blue; methylene blue and indigo blue; it can reduce nitrates to nitrites and ferric chloride to ferrous chloride. Let us, therefore, in the light of recent work not hesitate to bring tissue-respiration under the category of fermentation; its oxidative side is now generally admitted to be endo-enzymic, let us complete the conception and regard its reducing aspect as also endo-enzymic.